

binds to an HLA molecule and induces a cytotoxic T cell response, said method comprising steps of:

- (a) providing an amino acid sequence of an antigen of interest;
- (b) identifying within said sequence a putative T cell epitope, wherein said putative epitope consists of about 8-11 amino acid residues and is identified by the presence of an HLA-B7 structural supermotif associated with peptide binding to multiple HLA molecules, said structural supermotif comprising a first amino acid anchor residue at position two from the epitope's N-terminal residue, said first anchor residue selected from the group consisting of P, and a second anchor residue selected from the group consisting of V, I, L, F, M, W, Y, and A as the epitope's carboxyl-terminal amino acid residue;
- (c) preparing one or more peptide fragments of said antigen of interest that comprise the HLA B7 structural supermotif;
- (d) testing a first complex of said one or more motif-bearing peptide fragments and a first HLA molecule for an ability to be recognized by HLA-restricted cytotoxic T cells, and to thereby induce a cytotoxic T cell response to the epitope;
- (e) testing at least a second complex of said one or more motif-bearing peptide fragments and at least a second HLA molecule for an ability to be recognized by HLA-restricted cytotoxic T cells, and to thereby induce a cytotoxic T cell response to the epitope; and
- (f) selecting said one or more peptide fragments comprising the HLA-B7 structural supermotif that induce a cytotoxic T cell response to the epitope, when the epitope is in the first complex and when the epitope is in the at least a second complex.

68. The method of claim 67, wherein one of the peptide fragments has 8, 9, 10 or 11 residues.

69. The method of claim 67, wherein at least two peptide fragments are prepared.

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70. The method of claim 67, further comprising a step of determining binding affinity of said one or more peptide fragments for an HLA-B\*0701, HLA-B\*1401, HLA-B\*3501, HLA-B\*3503, HLA-B\*5101, HLA-B\*5301, HLA-B\*5401 or HLA-Cw6 molecule.

71. The method of claim 70, further comprising a step of identifying said one or more peptide fragments which has a binding affinity of an  $IC_{50}$  of less than about 500 nM for the HLA-B\*0701, HLA-B\*1401, HLA-B\*3501, HLA-B\*3503, HLA-B\*5101, HLA-B\*5301, HLA-B\*5401 or HLA-Cw6 molecule.

72. The method of claim 67, further comprising a step of:  
(g) determining binding affinity of said one or more peptide fragments for an HLA molecule that is not an HLA-B\*0701, HLA-B\*1401, HLA-B\*3501, HLA-B\*3503, HLA-B\*5101, HLA-B\*5301, HLA-B\*5401 or HLA-Cw6 molecule.

73. The method of claim 67, wherein the preparing step comprises isolation of the one or more peptide fragments from a natural source.

74. The method of claim 67, wherein the preparing step comprises synthesis of the one or more peptide fragments.

75. The method of claim 74, wherein the synthesis comprises chemical synthesis.

76. The method of claim 67, wherein the preparing step comprises expressing in a cell a recombinant nucleic acid molecule that encodes said one or more of the peptide fragments.

77. The method of claim 76, wherein the recombinant nucleic acid molecule encodes more than one peptide fragment.

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78. The method of claim 67, wherein the testing step occurs *in vitro*.
79. The method of claim 67, wherein the testing step occurs *in vivo*.
80. A method of making a HLA-B7 supermotif peptide that binds to an HLA molecule at an  $IC_{50}$  of less than about 500 nM, the method comprising steps of:
- (a) providing an amino acid sequence of an antigen of interest;
  - (b) identifying within said sequence a putative T cell epitope from the provided amino acid sequence, wherein said putative epitope consists of about 8-11 amino acid residues and is identified by the presence of an HLA-B7 structural supermotif associated with peptide binding to multiple HLA molecules, said structural motif comprising a first amino acid anchor residue at position two from the epitope's N-terminal residue, said first anchor residue selected from the group consisting of P, and a second amino acid anchor residue selected from the group consisting of V, I, L, F, M, W, Y, and A as the epitope's carboxyl-terminal amino acid residue;
  - (c) preparing one or more peptide fragments of said antigen of interest that comprise the HLA-B7 structural supermotif;
  - (d) contacting said one or more peptide fragments of step (c) with an HLA molecule; and,
  - (e) selecting said one or more peptide fragments that comprise an HLA-B7 structural supermotif that binds to an HLA molecule at a binding affinity of an  $IC_{50}$  of less than about 500 nM.
81. The method of claim 80, further comprising a step of:
- (f) contacting an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 restricted cytotoxic T lymphocyte with a complex of the peptide of step (e) and an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule, respectively.
82. The method of claim 80, wherein the contacting step occurs *in vitro*.

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83. The method of claim 80, wherein the contacting step occurs *in vivo*.

84. A method for making an immunogenic peptide that bears an HLA B7 supermotif and inducing an immune response, said peptide comprising an epitope consisting of about 8-11 residues that binds to multiple HLA molecules, and when bound to such an HLA molecule induce a cytotoxic T cell response, said method comprising steps of:

providing an amino acid sequence of an antigen of interest or a peptide fragment thereof, having an amino terminus and a carboxyl terminus;

identifying a putative T cell epitope within said amino acid sequence or a peptide fragment thereof, whereby said putative epitope comprises the structural B7 supermotif associated with peptide binding to multiple HLA molecules, said structural motif comprising a first amino acid residue at position two from an amino-terminal residue of the epitope, said first residue selected from the group consisting of P, and a residue selected from the group consisting of V, I, L, F, M, W, Y, and A at a carboxyl-terminal amino acid of the epitope;

obtaining at least one peptide fragment derived from the antigen that comprises the HLA-B7 structural supermotif;

testing a complex of said at least one peptide fragment and a first HLA molecule for an ability to be recognized by cytotoxic T cells restricted by the first HLA molecule and to thereby induce a cytotoxic T cell response to the epitope; and,

selecting said one or more peptide fragments comprising an HLA-B7 structural supermotif of the identifying step that induce a cytotoxic T cell response to the epitope.

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85. The method of claim 84, further comprising a step of:  
testing a complex of said at least one peptide fragment and at least a second HLA molecule for an ability to be recognized by cytotoxic T cells restricted by the at least a second HLA molecule and to thereby induce a cytotoxic T cell response to the epitope; and wherein the selecting step further comprises,

selecting said one or more peptide fragments comprising the HLA-B7 structural supermotif of the identifying step that induce a cytotoxic T cell response to the epitope when in complex with the first HLA molecule and when in complex with the at least a second HLA molecule.

86. The method of claim 84, wherein the providing step comprises providing and expressing nucleic acids that encode the amino acid sequence of the antigen of interest.

87. The method of claim 84, wherein the obtaining step comprises expressing a recombinant nucleic acid molecule that encodes the at least one peptide fragment.

88. The method of claim 87, wherein the obtaining step comprises expressing a recombinant nucleic acid molecule that encodes the at least one peptide fragment and one or more additional peptides; with a *proviso* that neither the at least one peptide fragment, the one or more additional peptides, nor any combination of the at least one peptide fragment and the one or more additional peptides comprise an entire native antigen.

89. The method of claim 84, wherein the obtaining step obtains the at least one peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

90. The method of claim 84, wherein the providing step comprises providing an amino acid sequence of an antigen of interest which is a cancer-associated antigen.

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91. The method of claim 90, wherein the providing step comprises providing an amino acid sequence from an antigen that is HER2/neu.

92. The method of claim 90, wherein the providing step comprises providing an amino acid sequence from an antigen that is p53.

93. The method of claim 90, wherein the providing step comprises providing an amino acid sequence from an antigen that is a MAGE antigen.

94. The method of claim 90, wherein the providing step comprises providing an amino acid sequence from an antigen that is a prostate antigen.

95. The method of claim 84, wherein the providing step comprises providing an amino acid sequence from an antigen that is derived from a pathogenic agent.

96. The method of claim 95, wherein the providing step comprises providing an amino acid sequence from an antigen that is HPV.

97. The method of claim 95, wherein the providing step comprises providing an amino acid sequence from an antigen that is HIV.

98. The method of claim 95, wherein the providing step comprises providing an amino acid sequence from an antigen that is HBV.

99. The method of claim 95, wherein the providing step comprises providing an amino acid sequence from an antigen that is HCV.

100. The method of claim 95, wherein the providing step comprises providing an amino acid sequence from an antigen that is a malaria antigen.

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101. The method of claim 84, wherein the obtaining step obtains a peptide fragment of 8, 9, 10 or 11 residues.

102. The method of claim 84, wherein the obtaining step obtains a peptide fragment of more than 11 residues, with a *proviso* that the fragment is not an entire native antigen.

103. The method of claim 84, wherein at least two peptide fragments are obtained.

104. The method of claim 84, further comprising a step of:  
determining binding affinity of an obtained peptide fragment for an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule.

105. The method of claim 104, further comprising a step of identifying an obtained peptide fragment that has a binding affinity of an  $IC_{50}$  for an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule of less than about 500 nM.

106. The method of claim 105, further comprising a step of identifying an obtained peptide fragment that has a binding affinity of an  $IC_{50}$  for an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule of less than about 125 nM.

107. The method of claim 106, further comprising a step of identifying an obtained peptide fragment that has a binding affinity of an  $IC_{50}$  for an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule of less than about 50 nM.

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108. The method of claim 84, wherein the obtaining step comprises isolation of the one or more peptide fragments from a natural source.

109. The method of claim 84, wherein the obtaining step comprises synthesis of the one or more peptide fragments.

110. The method of claim 109, wherein the synthesis comprises chemical synthesis.

111. The method of claim 84, wherein the testing step occurs *in vitro*.

112. The method of claim 84, wherein the testing step occurs *in vivo*.

113. A method for using a peptide fragment in accordance with claim 84, wherein:  
the testing step further comprises steps of complexing the at least one peptide fragment with an HLA molecule whereby a complex is created; and,  
contacting a CTL restricted by the HLA molecule with the complex of the provided peptide fragment and the HLA molecule, whereby a CTL response is induced.

114. A method for using a peptide fragment in accordance with claim 113, wherein:  
the testing step further comprises steps of complexing the at least one peptide fragment with an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule whereby a complex is created; and,  
contacting a CTL restricted by the HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule, respectively, with the complex of the provided peptide fragment and the HLA molecule, whereby a CTL response is induced.

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115. A method of making a peptide that bears an HLA B7 structural supermotif and binds to an HLA molecule at an  $IC_{50}$  less than about 500 nM, the method comprising steps:

providing an amino acid sequence or a peptide fragment thereof, having an amino terminus and a carboxyl terminus from an antigen of interest;

identifying a putative T cell epitope within the amino acid sequence or a peptide fragment thereof, whereby said putative epitope consists of about 8-11 residues and comprises the structural B7 supermotif associated with peptide binding to multiple HLA molecules, said supermotif comprising a first amino acid residue at a position two from an amino-terminal residue of the epitope, said first residue selected from the group consisting of P, and a residue selected from the group consisting of V, I, L, F, M, W, Y, and A as a carboxyl-terminal amino acid of the epitope;

obtaining at least one peptide fragment derived from the antigen of interest that comprises the HLA-B7 structural supermotif,

determining binding affinity of the at least one peptide for an HLA molecule;

and,

selecting a peptide having an  $IC_{50}$  of less than about 500 nM for the HLA molecule.

116. The method of claim 115, further comprising a step of:

contacting a cytotoxic T lymphocyte restricted by the HLA molecule with a complex of the selected peptide and the HLA molecule .

117. The method of claim 115, wherein the providing step comprises providing and expressing nucleic acids that encode the amino acid sequence of the antigen of interest.

118. The method of claim 115, wherein the obtaining step comprises providing and expressing nucleic acids that encode the at least one peptide.

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119. The method of claim 118, wherein the obtaining step comprises providing and expressing nucleic acids that encode the at least one peptide and at least one additional peptide, with a *proviso* that neither an additional peptide, nor a combination of an additional peptide and an at least one peptide fragment comprise an entire native antigen.

120. The method of claim 115, wherein the obtaining step comprises obtaining the at least one peptide fragment comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

121. The method of claim 115, wherein the providing step comprises providing an amino acid sequence derived from a cancer-associated antigen.

122. The method of claim 121, wherein the providing step comprises providing an amino acid sequence derived from an antigen that is HER2/neu.

123. The method of claim 121, wherein the providing step comprises providing an amino acid sequence derived from an antigen that is p53.

124. The method of claim 121, wherein the providing step comprises providing an amino acid sequence derived from an antigen that is a MAGE antigen.

125. The method of claim 121, wherein the providing step comprises providing an amino acid sequence derived from an antigen that is a prostate antigen.

126. The method of claim 115, wherein the providing step comprises providing an amino acid sequence from an antigen that is derived from a pathogenic agent.

127. The method of claim 126, wherein the providing step comprises providing an amino acid sequence derived from an agent that is HPV.

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128. The method of claim 126, wherein the providing step comprises providing an amino acid sequence derived from an agent that is HIV.

129. The method of claim 126, wherein the providing step comprises providing an amino acid sequence derived from an agent that is HBV.

130. The method of claim 126, wherein the providing step comprises providing an amino acid sequence derived from an agent that is HCV.

131. The method of claim 126, wherein the providing step comprises providing an amino acid sequence derived from an agent that is a malaria antigen.

132. The method of claim 115, wherein the obtaining step comprises obtaining a peptide of 8, 9, 10 or 11 amino acids in length.

133. The method of claim 115, wherein the obtaining step comprises obtaining a peptide of more than 11 amino acids in length, with a *proviso* that the peptide does not comprise an entire native antigen.

134. The method of claim 115, wherein the selecting step comprises selecting a peptide that comprises an  $IC_{50}$  for the HLA molecule of less than about 125 nM.

135. The method of claim 134, wherein the selecting step comprises selecting a peptide that comprises an  $IC_{50}$  for the HLA molecule of less than about 50 nM.

136. The method of claim 115, wherein the step of determining binding affinity comprises determining the binding affinity of the at least one peptide for an HLA molecule which is HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6.

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137. A method for using a selected peptide in accordance with claim 115, further comprising:

complexing the selected peptide with an HLA molecule whereby a complex is created; and,

contacting a CTL restricted by the HLA molecule with the complex of the selected peptide and the HLA molecule, whereby a CTL response is induced.

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138. A method for using a selected peptide in accordance with claim 137, further comprising:

complexing the selected peptide with an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule whereby a complex is created; and,

contacting a CTL restricted by the HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule, respectively, with the complex of the selected peptide and the HLA molecule, whereby a CTL response is induced.

139. A method of selecting a peptide that bears an HLA-B7 structural supermotif and which is immunogenic, said method comprising steps of:

a) identifying a putative epitope consisting of about 8-11 amino acid residues in an amino acid sequence, said epitope comprising an amino acid motif of P at a position two relative to an amino terminus of the epitope, and an residue selected V, I, L, F, M, W, Y, or A at a carboxyl terminus of the epitope;

b) obtaining a supermotif-bearing peptide derived from the amino acid sequence that comprises the putative epitope, with a *proviso* that the supermotif-bearing peptide is not an entire native antigen;

c) determining whether the supermotif-bearing peptide is immunogenic for cytotoxic T lymphocytes; and,

d) selecting a peptide of step c) that is immunogenic.

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140. The method of claim 139, wherein the obtaining step comprises expressing a nucleic acid sequence that encodes the peptide.

141. The method of claim 140, wherein the obtaining step comprises expressing a nucleic acid sequence that encodes the supermotif-bearing peptide and one or more additional peptides, with a *proviso* that neither an additional peptide, nor any combination of a one or more additional peptides and the supermotif-bearing peptide comprise an entire native antigen.

142. The method of claim 139, wherein the obtaining step comprises obtaining the supermotif-bearing peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

143. The method of claim 139, wherein the obtaining step comprises isolation of the supermotif-bearing peptide from a natural source.

144. The method of claim 139, wherein the obtaining step comprises synthesis of the supermotif-bearing peptide.

145. The method of claim 144, wherein the synthesis comprises chemical synthesis.

146. The method of claim 139, wherein the obtaining step comprises obtaining a supermotif-bearing peptide of 8, 9, 10 or 11 amino acids in length.

147. The method of claim 139, wherein the obtaining step comprises obtaining a supermotif-bearing peptide of more than 11 amino acids in length, with a *proviso* that the supermotif-bearing peptide does not comprise an entire native antigen.

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148. The method in accordance with claim 139, wherein the step of determining whether the supermotif-bearing peptide is immunogenic comprises complexing the motif-bearing peptide and an HLA molecule whereby a complex is created, and contacting a CTL restricted by the HLA molecule with the complex, whereby a CTL response is induced.

149. The method in accordance with claim 139, wherein the step of determining whether the motif-bearing peptide is immunogenic occurs *in vivo* or *in vitro*.

150. A method for using a selected peptide in accordance with claim 139, further comprising:

e) complexing the selected peptide with an HLA molecule whereby a complex is created; and,

f) contacting a CTL restricted by the HLA molecule with the complex of the selected peptide and the HLA molecule, whereby a CTL response is induced.

151. A method for using a selected peptide in accordance with claim 150, wherein steps e) and f) further comprise:

complexing the selected peptide with an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule whereby a complex is created; and,

contacting a CTL restricted by the HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule, respectively, with the complex of the selected peptide and the HLA molecule, whereby a CTL response is induced.

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152. A method of selecting a peptide that bears an HLA B7 supermotif, and binds to an HLA molecule at a level of affinity predicted to be immunogenic in humans, said method comprising steps of:

- a) obtaining a peptide comprising an epitope consisting of about 8-11 amino acid residues, said epitope comprising an amino acid P at a position two relative to an amino terminus of the epitope, and V, I, L, F, M, W, Y or A at a carboxyl terminus of the epitope;
- b) determining binding affinity of the peptide for an HLA molecule; and,
- c) selecting a peptide of step b) that comprises an  $IC_{50}$  for the HLA molecule of less than about 500 nM.

153. The method of claim 152, wherein the selecting step comprises selecting a peptide of step b) that comprises an  $IC_{50}$  for the HLA molecule of less than about 125 nM.

154. The method of claim 153, wherein the selecting step comprises selecting a peptide of step b) that comprises an  $IC_{50}$  for the HLA molecule of less than about 50 nM.

155. The method of claim 152, wherein the obtaining step comprises expressing a nucleic acid sequence that encodes the peptide.

156. The method of claim 155, wherein the obtaining step comprises expressing a nucleic acid sequence that encodes the peptide and one or more additional peptides, with a *proviso* that neither an additional peptide, nor any combination of a one or more additional peptides and the peptide comprise an entire native antigen.

157. The method of claim 152, wherein the obtaining step comprises obtaining the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

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158. The method of claim 152, wherein the step of determining binding affinity of the peptide for an HLA molecule determines the affinity for an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule.

159. A method for using a selected peptide in accordance with claim 152, further comprising:

d) complexing the selected peptide with an HLA molecule whereby a complex is created; and,

e) contacting a CTL restricted by the HLA molecule with the complex of the selected peptide and the HLA molecule, whereby a CTL response is induced.

160. A method for using a selected peptide in accordance with claim 159, wherein the steps e) and f) further comprise:

complexing the selected peptide with an HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule whereby a complex is created; and,

contacting a CTL restricted by the HLA-B0701, HLA-B1401, HLA-B3501, HLA-B3503, HLA-B5101, HLA-B5301, HLA-B5401 or HLA-Cw6 molecule, respectively, with the complex of the selected peptide and the HLA molecule, whereby a CTL response is induced.

161. The method of claim 152, wherein the obtaining step comprises isolation of the peptide from a natural source.

162. The method of claim 152, wherein the obtaining step comprises synthesis of the peptide.

163. The method of claim 162, wherein the synthesis comprises chemical synthesis.

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